# SUPPLEMENTS

## The Ability Of Nitric Oxide To Lower Intraocular Pressure Is Dependent On Soluble Guanylate Cyclase

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**Supplemental Figures and Tables:**

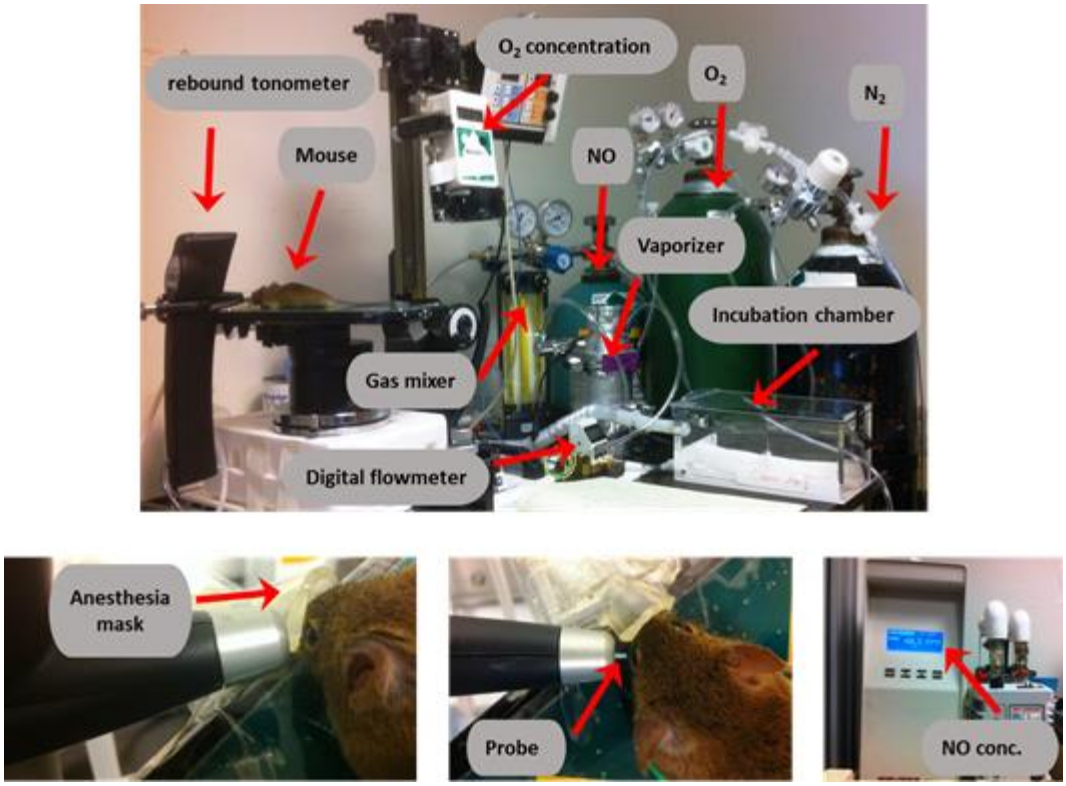



**Figure S1: Anesthetized and Awake IOP Measurement Setup. Awake Study:** Rebound tonometry (Icare, Vantaa, Finland) was used to measure IOP in anesthetized mice at baseline and 10 minutes after breathing either NO or control gas. Because isoflurane itself has IOP lowering capabilities, we also measured IOP values awake WT and GC-1-/- mice breathing NO or control gas. Prior to the study, mice were acclimated to manual handling for 3 weeks. During the first week, animals were acclimated to manual handling via holding them by the scruff of their neck for 5-10 min daily. The following week, animals were familiarized with the probe of the rebound tonometer. Murine eyes were topically anesthetized by Proparacaine Hydrochloride (0.5 %) (Bausch+Lomb) eye drops (one drop per eye). After an incubation of 10 min, mice were

manually restrained (grasping the scruff of the neck close to the base of the skull between the thumb and forefinger) and exposed to the rebound tonometer. The last two days, animals didn't receive any eye drops. During the third and last week, the animals were placed in the incubation chamber (World Precision Instruments, Sarasota, FL USA) and exposed to O2 (100 %). At predefined time points (0, 40 min) animals were removed, restrained and their IOP was measured. After 3 weeks of training, animal behavior remarkably improved and experiments were conducted. Mice were placed in an anesthesia induction chamber and exposed to either 40 ppm NO in 90% O2/10% N2 or the control gas via a gas inlet at a flow rate of 1000 ml/minute. A gas outlet was open to the environment to maintain the induction chamber at ambient pressure. IOP was measured at baseline and 40 minutes after starting the treatment.

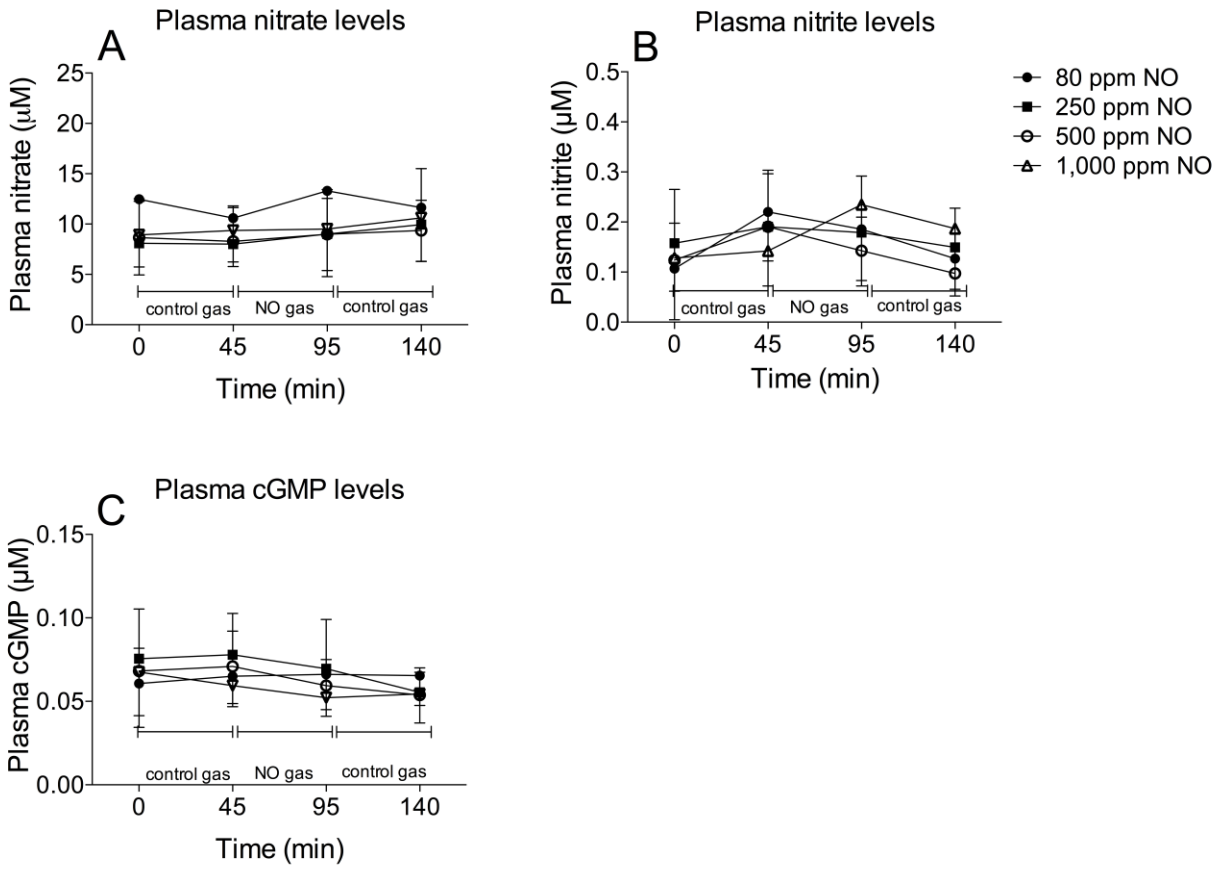



**Figure S2: NO metabolites and cGMP levels in lamb plasma before and after treatment with topical NO.** (A) Plasma nitrate, (B) nitrite, and (C) cGMP levels at baseline, when delivering the control gas, and after NO exposure (80, 250, 500, 1,000 ppm; n=5, P>0.05).

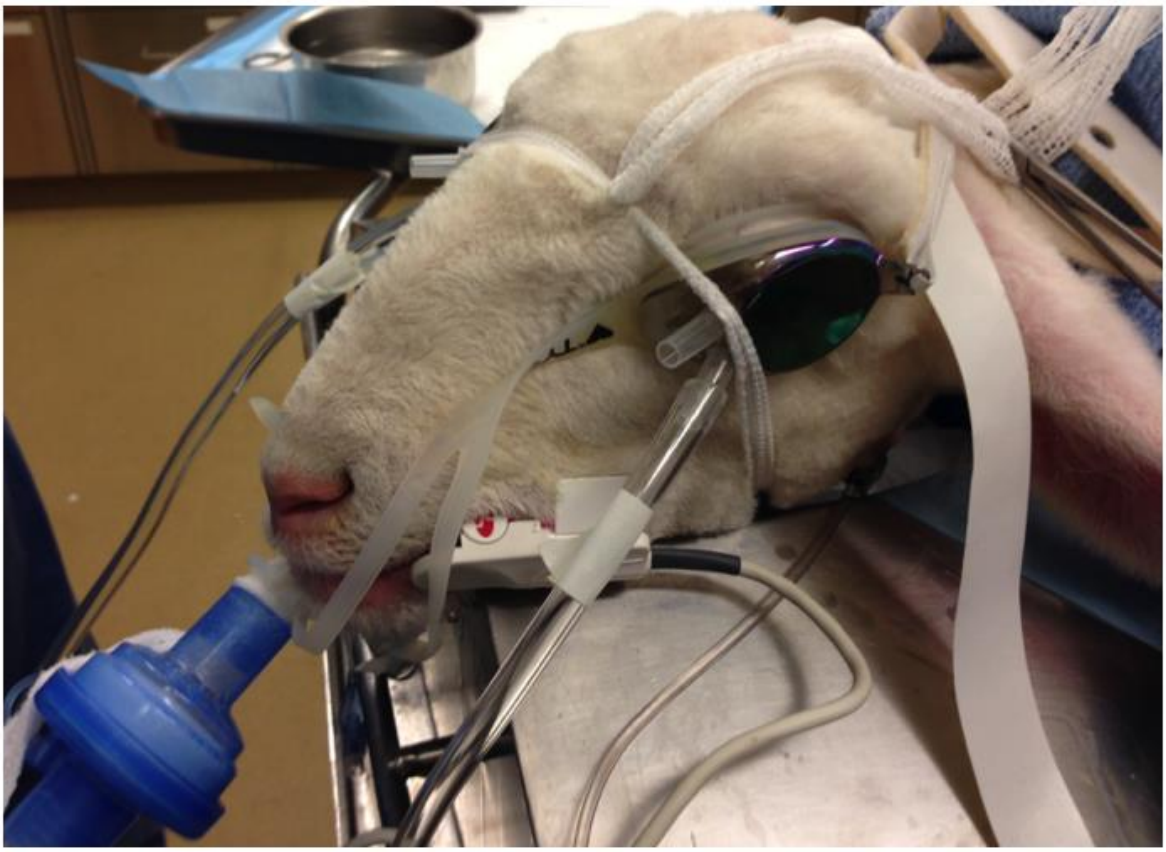
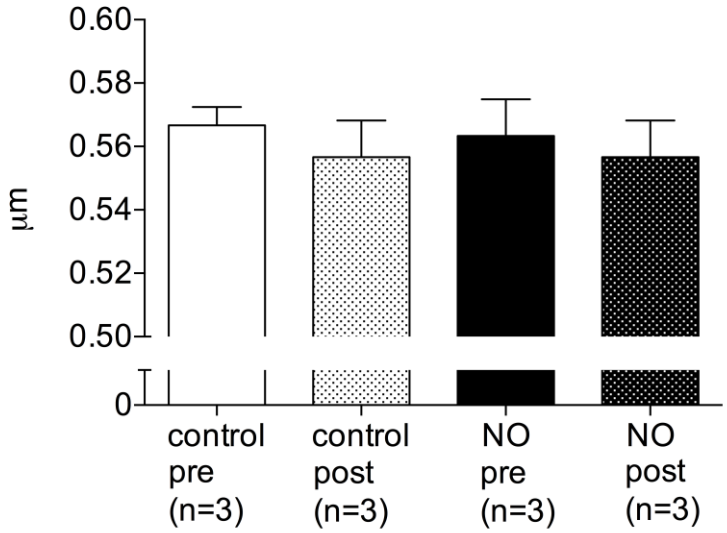


**Figure S3: NO gas delivery device for topical NO application.** Anesthetized and mechanically ventilated lamb wearing the device. The device consists of regular swimming goggles that are placed over the eyes. Sidewise, two holes were drilled (gas inlet and outlet) into the plastic and hose barbs were glued onto the wholes. NO gas flow of approximately 500ml/min was applied. To keep the gas chamber at ambient pressure, the gas outlet remained open to the atmosphere. The eyes were in direct contact with the NO gas and the device prevented the dilution of NO gas with room air. As the cornea is avascular and requires exposure to room air to gain oxygen, we added 10% oxygen to the gas mixture when exposing the cornea to NO gas (which is balanced in nitrogen). The presence of oxygen and the high concentration of NO (up to 1,000 ppm) increased the likelihood of nitrogen dioxide formation, a potentially harmful

byproduct as nitrogen dioxide may react with water to form nitric acid which causes severe tissue damage. Thus, the gas mixture (1,000ppm NO/$N_2$ +10% $O_2$) passed a customized filter containing calcium hydroxide to remove nitrogen dioxide prior to the delivery to the cornea.

**Figure S4: Corneal thickness before and after exposure of 1,000 ppm NO to the lamb's cornea**




Ovine central corneal thickness was measured before and after a 1 h exposure to 1,000 ppm NO using ultrasound: three- to four month old lambs have a central corneal thickness of approximately 570 µm, similar to what is typically observed in humans (Figure 5A). The corneal thickness was similar before and after exposure to 1,000 ppm NO and the depth of the cornea did not differ in NO-treated versus control-gas treated eyes.

**Table S1: Hemodynamic parameters before, during and after exposure of 1,000 ppm NO to the lamb's cornea.**

| | control gas (n=6) | NO exposure (n=6) | after NO treatment (n=6) | p-value |
|---|---|---|---|---|
| HR (bpm) | 105±14 | 103±14 | 100±13 | 0.80 |
| MAP (mmHg) | 69±9 | 71±9 | 69±7 | 0.91 |
| CVP (mmHg) | 2±0.8 | 2±0.8 | 2±0.7 | 0.98 |
| SVRI ($dyn \cdot sec \cdot m^2 \cdot cm^{-5}$) | 858±123 | 945±207 | 953±179 | 0.59 |
| PAP (mmHg) | 12±1 | 12±1 | 12±1 | 0.58 |
| PVRI ($dyn \cdot sec \cdot m^2 \cdot cm^{-5}$) | 98±12 | 101±14 | 105±11 | 0.68 |
| PCWP (mmHg) | 5±1 | 4±1 | 4±1 | 0.81 |
| CI ($l \cdot min^{-1} \cdot m^{-2}$) | 6.3±0.9 | 5.9±0.8 | 5.8±0.7 | 0.59 |

No differences were observed between control-gas treatment, NO exposure and after the NO treatment in terms of heart rate (HR), systemic mean arterial pressure (MAP), central venous pressure (CVP), systemic vascular resistance index (SVRI), mean pulmonary arterial pressure (PAP), pulmonary vascular resistance index (PVRI), pulmonary capillary wedge pressure (PCWP) and cardiac index (CI).